

Effect of Clenbuterol on Growth and Body Composition During Food Restriction in Rats

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ABSTRACT: Clenbuterol was administered as a dietary admixture (4 mg/kg diet) to three groups of male Wistar rats ($n = 8$) housed individually in metabolism cages and fed for 15 d at 110, 160, and 235% (ad libitum) of estimated requirement for energy maintenance. Untreated groups at each level of energy intake were also included. There was no effect of clenbuterol on food intake in the ad libitum group, but the drug produced significant increases in body weight, feed efficiency, and carcass weight, dressing and protein content at all three levels of energy intake. This effect of clenbuterol was particularly noticeable in the restricted animals. Clenbuterol

caused changes in body composition (increased percentage of water and protein, decreased percentage of fat) in the ad libitum rats but had no effect in the restricted groups. The reduction in the growth of the viscera caused by energy restriction was not affected by clenbuterol, apart from in the 110% restricted group, where the gastrointestinal tract was 26% heavier in the clenbuterol-treated rats. The results show that the growth anabolic actions of clenbuterol can be sustained and may be even more marked in rats fed restrictively than in those given ad libitum access to feed.

Key Words: Energy Restriction, Food Intake, Carcass Composition, Anabolism, Beta-Adrenergic Agonists

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Introduction

It has been shown that the selective β_2 -adrenergic agonist clenbuterol has anabolic and carcass repartitioning effects in rats (Emery et al., 1984; Reeds et al., 1986; Rothwell and Stock, 1987b) and in farm animals (Dalrymple and Ingle, 1987; Scholtyssek, 1987; Buayne et al., 1991). These effects are mainly due to increases in body protein (mainly skeletal muscle) and decreases in fat content. The effects of clenbuterol and similar repartitioning agents (e.g., cimaterol) in these and other studies concerned with its mode of action (e.g., Beermann et al., 1987; Kim et al., 1987; Zeeman et al., 1988) have always been observed in laboratory and farm animals under ad libitum intake conditions. The only exception is the

study by Choo et al. (1990), who studied the effects of clenbuterol on weight gain and muscle weight and protein content in rats fed 50% ad libitum intake for 7 d. To date, the effects on body composition and other parameters in food-restricted animals have not been studied systematically.

For various political, economic, and other reasons, the use of anabolic agents in meat production is no longer allowed in certain countries, although they may be used for clinical, veterinary purposes, and there is growing interest in the potential use of anabolic β_2 -adrenergic agonists in the treatment of various human muscle-wasting conditions (Maltin et al., 1993). Muscle wasting also occurs naturally in livestock suffering from seasonal decreases in food availability, and in many parts of the world protein loss is a major constraint on animal production, as emphasized by Touchberry (1967), Williamson and Payne (1980), and Preston and Leng (1987). These authors reported livestock weight losses of 25% to 40% during dry seasons in tropical and subtropical areas. Methods for minimizing this seasonal decrease in live weight and the loss of protein should therefore be of interest, and it was decided to study the effects of clenbuterol on growth and on body composition.

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Materials and Methods

Adult male (200 g) rats were obtained from the Wistar colony maintained at the Gulbenkian Foundation Science Institute (Lisbon, Portugal) and divided into seven weight-matched groups ($n = 8$). One group served as a baseline group (**B0**) that was killed for analysis of initial body composition, and the other groups were allocated to three dietary regimens (two groups per regimen). One dietary regimen involved allowing the rats to feed ad libitum, and the other two involved restricting energy intake to 160% and 110% of estimated maintenance requirements. Maintenance digestible energy was assumed to be 460 kJ/(kg^{.75}.d) (NRC, 1978), and by using the manufacturer's values for the metabolizable energy density of the stock diet (CRM, Biosure Ltd., U.K., metabolizable energy density 12.8 kJ/g, 17.5% CP, 2.4% fat, 5.3% ash), 160% and 110% of maintenance was calculated to be provided by feeding 17 and 12 g/d per rat, respectively. This amount was then fed throughout the 15-d experiment.

Within each feeding regimen, one group received the diet and the other group received clenbuterol (Boehringer Ingleheim, Bracknell, U.K.) incorporated into the diet at a concentration of 4 mg/kg. Thus, there were six experimental groups, designated as follows: ad libitum \pm clenbuterol (**ADL**, **ADLC**); 17 g restricted \pm clenbuterol (**R17**, **R17C**); 12 g restricted \pm clenbuterol (**R12**, **R12C**). The rats were housed individually in metabolism cages in a room kept at $24 \pm 1^\circ\text{C}$, 60 to 65% humidity and with a 12-h light:dark cycle. Food intake (corrected for spillage) was recorded daily and body weight every other day.

On d 0 (B0 group) and d 15 (all experimental groups) the rats were anesthetized (sodium pentothal) and killed by cardiac puncture and exsanguination. Heart, liver, kidneys, gastrocnemius, and soleus muscles and gastrointestinal tract were dissected, cleaned, weighed, and frozen (-20°C) until analyzed. The eviscerated carcass was cleaned and "dressed" (decapitation, amputation at the tibio-tarsal, radio-cubit-carpal joints, skinning and interscapular brown adipose tissue dissection) before weighing and freezing. The dry mass of carcass and isolated muscles and organs was determined by freeze-drying (Modulyo, Edwards, U.K.), and after homogenization the protein content of all samples was determined by Kjeldahl digestion (Buchi 315, Schweiz, Germany). Skin weight was determined with hair. The fat and ash content of the samples was taken to be equal to the difference between the carcass dry mass and protein content, and it was assumed that changes in lipid content would account for most, if not all, of the experimental variation seen in this derived measurement (Fuller et al., 1990).

All results have been presented as mean values, \pm SE, and were subjected to ANOVA to determine the effect of feeding level, clenbuterol treatment, and their

interaction. Post hoc significant differences between various treatment groups have been identified by the least significant difference (LSD) test ($P < .05$). Within each feeding level, significant differences between untreated and clenbuterol-treated rats were determined using Student's *t*-test for unmatched data; all probabilities quoted are two-tailed.

Results

In the three experimental diets there were no significant differences in the mean food intake as a result of treatment with clenbuterol (Table 1).

The energy intake of the rats with ad libitum access to feed was equivalent to 235% of maintenance requirements, compared to the fixed intake of 160% and 110% of maintenance in the R17 and R12 groups. At all levels of food intake, clenbuterol treatment resulted in greater body weight gains, and over the course of the experiment the gain was increased by 37% and 49%, respectively, in the ADLC and R17C groups. The most severely restricted rats (R12) lost weight, but the effect of clenbuterol was to reverse this and produce a 25-g gain in the R12C rats. The effect of clenbuterol on weight gain was more noticeable during the first week, but differences in the rate of gain were still seen up to the end of the experiment (data not shown). The overall gain (as a percentage of initial body weight) is shown for each group in Figure 1, where the effects of both energy restriction and clenbuterol treatment can be seen clearly. Feed efficiency (i.e., weight gain/food intake) could not be calculated for the R12 group because the rats lost weight, whereas the R12C rats had a feed efficiency that was similar to that of the untreated R17 group. At this higher level of food intake (17 g/d), clenbuterol produced a significant 51% increase in feed efficiency, which compares with the 26% increase seen in the ad libitum rats.

In Table 1, the cleaned carcass weight has been expressed as a percentage of total body weight (i.e., "dressing%"), and when compared to the untreated values was found to be increased by 17%, 14%, and 5% in the R12C, R17C, and ADLC groups, respectively. This effect of clenbuterol was seen to be even more pronounced, when considering the gain in carcass weight, with 40% and 70% increases seen in the ADLC and R17C groups, respectively, and a more than threefold increase in the most severely restricted group (R12C). In terms of carcass composition, clenbuterol treatment in rats with ad libitum intake induced significant increases in percentage of water and protein, and a decrease in carcass fat, although it should be emphasized that the fat values include the mineral content of the carcass. Compared to the ad libitum rats, the effect of energy restriction at both levels was to increase the percentage of water and protein in the carcass and to decrease the fat.

Table 1. Food intake, growth, and carcass parameters^a

Item	B0 ^b	R12	R12C	R17	R17C	ADL	ADLC	LSD ^c	F-test ^d		
									Diet	Trt.	Diet × Trt.
Food intake, g	164.8 (8.3)	175.7 (2.8)	178.7 (.5)	252.7 (1.1)	248.0 (1.4)	363.6 (9.5)	379.3 (6.8)	30.3	171.4 ^z	.26	.504
Final body wt, g	195.2 (9.7)	200.9 (3.1)	219.8*** (2.3)	239.3 (2.2)	263.0*** (3.8)	286.6 (9.4)	322.1* (12.3)	19.3	96.4 ^z	22.1 ^z	.798
Weight gain, g	—	−.6 (2.1)	25.4*** (3.2)	37.8 (2.4)	56.3*** (3.5)	97.6 (5.2)	128.4** (7.4)	12.5	272.9 ^z	49.2 ^z	1.008
Gain intake, % ^e	—	—	14.2 (1.3)	15.0 (1.5)	22.7*** (1.6)	26.8 (1.7)	33.8*** (1.8)	—	—	—	—
Carcass, dressing, %	42.23 (1.8)	50.00 (.6)	58.3*** (.3)	50.8 (.3)	57.7*** (.5)	50.4 (.5)	53*** (.9)	1.5	15.8 ^z	187.9 ^x	16.8 ^z
Gain, %	—	12.9 (1.3)	42.4 (2.0)	35.1 (1.8)	59.6 (.9)	61.0 (3.0)	85.3 (4.5)	7.4	156.9 ^z	153.2 ^z	.647
Water, %	69.71 (.91)	73.4 (.3)	73.6 (.4)	71.2 (.5)	72.8 (.8)	67.3 (.8)	71.0 (.6)	1.5	35.5 ^z	20.2 ^z	6.05
Protein, %	16.3 (.4)	19.4 (.2)	19.7 (.3)	19.2 (.4)	20.3 (.2)	17.0 (.3)	18.3* (.4)	.8	28.3 ^z	.7	6.6 ^y
Lipid, %	17.2 (.5)	7.2 (.2)	6.7 (.2)	8.7 (.4)	7.9 (.4)	15.6 (.9)	10.5*** (.5)	1.5	81.8 ^z	26.2 ^z	12.9 ^z

^aValues are in means (SEM, n = 8). **P* < .05; ***P* < .01; ****P* < .001 vs respective control group (Student's unpaired *t*-test).
^bB0 values were not subject to ANOVA.
^cLSD = least significant difference between groups (ANOVA).
^dFor *F*-test results ^y*P* < .01; ^z*P* < .001 for *F* ratio significance levels.
^eGain/intake values only subject to Student's unpaired *t*-test.

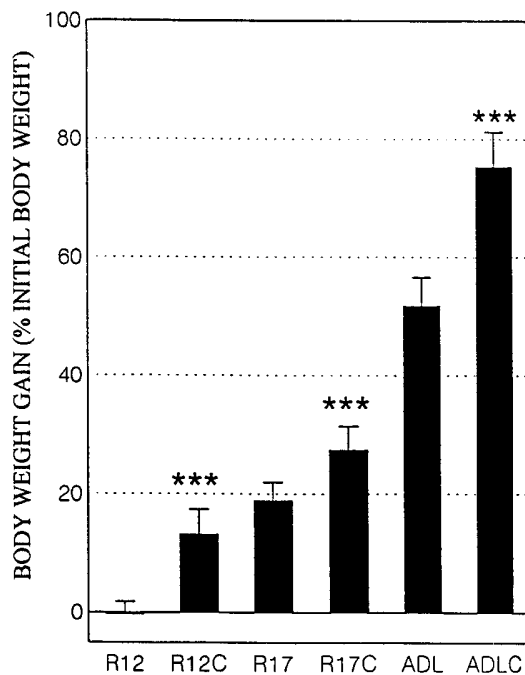


Figure 1. Effect of food restriction and clenbuterol treatment on weight gain. Mean values (n = 8); bar = SE; ****P* < .001 vs respective control group (unpaired *t*-test).

Clenbuterol treatment had no effect on this response to energy restriction.

The weights of the principal organs at the end of the experiments are shown in Table 2. Food restriction significantly reduced the weights of the studied organs. In the ad libitum groups, clenbuterol produced a significant increase in the weight of the heart, gastrocnemius, and soleus muscles and brown adipose tissue (**BAT**) and a significant decrease in the weight of the epididymal fat pad. In rats fed the restricted diets, clenbuterol induced the decrease in kidney weight in the R12C group and epididymal fat pad of the R17C group. In the most severely restricted group (R12) clenbuterol protected the gastrointestinal tract and BAT from weight loss. As a result, the gastrointestinal tract in the R12C group was 26% heavier than that in the R12 group, and BAT was 23% greater. The most obvious effects of clenbuterol treatment were on the skeletal muscles, where at both restriction levels the mass of the muscle was significantly greater than in the untreated rats. For gastrocnemius, the increases were 28% (R17C) and 38% (R12C), and for soleus the increases were 47% (R17C) and 44% (R12C); these compare with 25% (gastrocnemius) and 40% (soleus) in the ADLC groups. A rather unexpected finding was the smaller mass (24% decrease) of skin in the clenbuterol-treated restricted groups.

Table 2. Organ weights^a

Item	B0 ^b	R12	R12C	R17	R17C	ADL	ADLC	LSD ^c	F-test ^d		
									Diet	Trt.	Diet × Trt.
Heart, g	.67 (.04)	.63 (.01)	.63 (.01)	.70 (.01)	.74 (.02)	.86 (.02)	1.08*** (.03)	.06	147.1 ^x	23.3 ^x	16.5 ^x
Kidney, g	1.66 (.16)	1.69 (.06)	1.50* (.04)	1.94 (.04)	1.90 (.06)	2.10 (.06)	2.28 (.08)	.17	50.8 ^x	.09	5.2 ^z
Liver, g	8.99 (.71)	5.98 (.10)	5.75 (.12)	7.25 (.19)	7.15 (.18)	12.63 (.64)	12.87 (.86)	2.20	122.9 ^x	.38	.522
GIT, g ^e	1.90 (.22)	1.02 (.02)	1.29** (.09)	1.53 (.07)	1.53 (.05)	2.52 (.19)	2.85 (.36)	.13	40.7 ^x	1.9	.49
Gastrocnemius, g	1.54 (.09)	2.15 (.05)	2.96*** (.07)	2.69 (.10)	3.43*** (.05)	5.57 (.07)	3.42*** (.14)	.25	19.6 ^x	126.0 ^x	.211
Soleus, g	.27 (.02)	.41 (.2)	.59*** (.01)	.47 (.01)	.69*** (.01)	.50 (.04)	.70*** (.03)	.06	11.5 ^x	113.1 ^x	.176
BAT, g ^f	1.89 (.12)	.26 (.01)	.32** (.01)	.39 (.03)	.40 (.01)	.69 (.06)	.85* (.07)	.11	86.3 ^x	5.8 ^z	1.87
WAT, g ^g	1.38 (.03)	1.10 (.02)	1.06 (.07)	2.39 (.11)	1.79*** (.14)	4.47 (.41)	3.18*** (.30)	.64	76.1 ^x	12.3 ^y	3.8 ^z
Skin, g	24.45 (2.5)	28.31 (.91)	21.2*** (.56)	36.15 (.73)	28.50*** (.42)	34.83 (1.22)	36.08 (1.73)	2.95	57.0 ^x	29.5 ^x	11.9 ^x

^aValues are in means (SEM, n = 8). **P* < .05; ***P* < .01; ****P* < .001 vs respective control group (Student's unpaired *t*-test).

^bB0 values were not subject to ANOVA.

^cLSD = least significant difference between groups (ANOVA).

^dFor *F*-test results ^x*P* < .05; ^y*P* < .01; ^z*P* < .001 for *F* ratio significance levels.

^eGIT = gastrointestinal tract.

^fBAT = interscapular brown adipose tissue.

^gWAT = epididymal fat pad.

The protein content (percentage of dry weight) of the main viscera is shown in Table 3, where it will be seen that there were no changes in protein concentration as a result of food restriction, except for a significant increase in liver and in gastrointestinal tract. In the restricted R12 group, clenbuterol induced an increase in protein concentration in the heart and a decrease of this parameter in the kidney. In the ad libitum rats clenbuterol also decreased percentage of protein in the heart. The other significant changes caused by clenbuterol in organ protein concentration were seen in R12 and ad libitum rats with, respectively, 23 and 13% increases in the gastrointestinal tract.

These changes (or lack of change) in composition do not take account of differences in organ size, and in Table 4 the total protein content of heart, kidney, liver, and gastrointestinal tract has been presented along with the total carcass protein content. Determination of the protein content of the two skeletal muscles (gastrocnemius and soleus) was not performed because the carcass protein effectively represents muscle protein. The carcass protein was not affected by modest food restriction (R17) but was reduced by the R12 level of feeding. Food restriction significantly decreased protein content in all the other organs studied. Clenbuterol treatment produced sig-

nificant 19 and 16% increases in carcass protein content in, respectively, the ADLC and R17C groups, and a 23% increase in the R12C group. Total protein content in the hearts of the ADLC rats was increased. Perhaps the most notable changes were the respective 30 and the 46% increases in total protein content of the liver and the gastrointestinal tract in the R12C rats.

Discussion

There are a few reports in the literature that show effects of chronic treatment with clenbuterol on food intake. Emery et al. (1984) injected rats twice daily with 2 mg/kg clenbuterol and found that food intake was increased, whereas Hanrahan et al. (1986) in studies in poultry and pigs found that food intake was decreased when doses exceeded .01 mg/kg and .03 mg/kg, respectively, in the ration. However, most authors report no effect of clenbuterol on food intake, and Reeds et al. (1988), using the same dose used here (4 mg/kg diet), found no effect, as did Sainz and Wolff (1986), who used much higher doses of clenbuterol (25 to 150 mg/kg of diet). Even when clenbuterol is injected, thereby producing higher peak plasma levels, there is little effect on food intake (Rothwell and

Table 3. Organ protein content^a

Item	R12	R12C	R17	R17C	ADL	ADLC	LSD ^b	F-test ^c		
								Diet	Trt.	Diet × Trt.
Heart	17.68 (.46)	18.55* (.26)	17.49 (.59)	18.16* (.18)	17.14 (.32)	16.55* (.34)	.96	6.05	1.00	2.16
Kidney	17.42 (.21)	16.93* (.21)	16.68 (.69)	16.90 (.26)	16.89 (.15)	16.88 (.25)	1.02	.66	.09	.54
Liver	22.82 (.24)	22.80 (.31)	22.60 (.80)	22.40 (.26)	18.18 (.38)	18.42 (.65)	1.43	52.3 ^x	2.0	.10
GIT ^d	72.32 (1.79)	89.02*** (2.77)	62.95 (1.85)	63.77 (1.03)	48.37 (1.28)	54.52* (1.93)	5.43	125.6 ^x	22.5 ^x	11.6 ^x

^aValues are in means (SEM, n = 8). **P* < .05; ****P* < .001 vs respective control group (Student's unpaired *t*-test).

^bLSD = least significant difference between groups (ANOVA).

^cFor *F*-test results ^x*P* < .001 for *F* ratio significance levels.

^dGIT = gastrointestinal tract.

Stock 1987a,b). Thus, it can be concluded, as others have done before, that the anabolic effects of clenbuterol do not depend on an increase in food intake. Moreover, this study shows that the effect is sustained even when food intake is restricted to near maintenance levels.

Although the effects of clenbuterol on weight gain were still observed during the last (second) week of the experiment, most of the effects were seen in the first week. This agrees with other studies in rats in which most of the anabolic effect had reached a maximum at about 8 to 10 d and, thereafter, treated and untreated rats grew at similar rates (Reeds et al., 1988). The severely restricted rats (R12) might be an exception to this, because differences between treated and untreated rats persisted up to the end of the 15-d experiment. In fact, the overall effects of clenbuterol were much more noticeable in the severely restricted group, and although feed efficiency was significantly increased in the ad libitum and in the

R17 groups (26% and 51%, respectively), the effect of clenbuterol in the R12 group was to reverse the weight loss at this level. This resulted in a positive feed efficiency that was comparable (14%) to that observed in the untreated R17 group, which had consumed 40% more food.

Work on farm animals has shown increases in carcass dressing following clenbuterol treatment. Dalrymple and Ingle (1987) found significant increases in percentage dressing in broilers and lambs but not in steers, whereas Williams et al. (1987) found significant increases in dressing in veal calves. In the present study, clenbuterol produced a significant increase in dressing in the rats with ad libitum feed intake, but the effect was greater in the restricted groups and highest in the most restricted group (R12). In this group, the improvement in dressing percentage produced by clenbuterol was three times greater than the improvement in the ad libitum rats.

Table 4. Total protein content of the carcass and major viscera

Item	B0 ^b	R12	R12C	R17	R17C	ADL	ADLC	LSD ^c	F-test ^d		
									Diet	Trt.	Diet × Trt.
Carcass, g	13.4 (1.6)	19.09 (.36)	23.43*** (.28)	23.27 (.45)	27.15*** (.53)	23.09 (.58)	27.59*** (1.01)	1.68	31.0 ^x	77.8 ^x	.15
Heart, g	.111 (.006)	.110 (.004)	.118 (.003)	.124 (.003)	.133 (.006)	.148 (.007)	.180* (.006)	.015	47.5 ^x	13.2 ^y	3.2
Kidney, g	.268 (.017)	.292 (.014)	.259 (.008)	.328 (.010)	.323 (.010)	.352 (.014)	.385 (.016)	.037	26.9 ^x	.02	3.3
Liver, g	1.42 (.13)	1.01 (.01)	1.32*** (.03)	1.74 (.09)	1.61 (.05)	2.29 (.14)	2.36 (.22)	.34	49.4 ^x	.69	1.75
GIT, g ^e	1.01 (.23)	.74 (.02)	1.08** (.16)	.97 (.03)	.99 (.02)	1.23 (.10)	1.20 (.14)	.29	4.9 ^z	1.79	1.84

^aValues are in means (SEM, n = 8). **P* < .05; ***P* < .01; ****P* < .001 vs respective control group (Student's unpaired *t*-test).

^bB0 values were not subject to ANOVA.

^cLSD = least significant difference between groups (ANOVA).

^dFor *F*-test results ^x*P* < .05; ^y*P* < .01; ^z*P* < .001 for *F* ratio significance levels.

^eGIT = gastrointestinal tract.

Carcass composition was determined by direct analysis of dry matter content (i.e., water by difference) and protein (Kjeldahl digestion), but it should be emphasized that the fat values presented in the results include the ash content of the carcass (see Methods). It has been argued (Fuller et al., 1990) that this approach provides a reliable index of variations in lipid content, even though absolute values for fat will be overestimated. The reductions in carcass fat following energy restriction may have been slightly exaggerated by a simultaneous decrease in the mineral content of carcass bone, but it can be assumed that most of the loss in this component was due to mobilization of fat to meet energy requirements. The decreases in fat were accompanied by increases in the percentages of water and protein. However, clenbuterol treatment failed to influence these changes in composition following food restriction, and only in the ad libitum animals where there were significant increases in water and protein, which were accompanied by a simultaneous decrease in carcass percentage of fat.

Changes in percentage body composition following clenbuterol treatment have been noticed by other workers (e.g., Dalrymple et al., 1987; Rothwell and Stock, 1987a), the most noticeable changes occurring in carcass water. However, the anabolic effects of clenbuterol need not necessarily result in an increase in percentage of water or protein in the carcass because there is also an increase in carcass mass. The results obtained here are consistent with all previous reports of significant increases in protein deposition following clenbuterol treatment in animals given ad libitum access to feed. The highest increase in carcass protein (23%) was shown in the severely restricted treated rats (R12C). This is another illustration of the enhanced anabolic effect of clenbuterol in the highly restrictively fed animals.

Because the carcass protein represents that contained almost entirely in muscle, it is not surprising to find that leg muscle shows similar responses to clenbuterol treatment; the mass of gastrocnemius and soleus muscles was increased 25 and 40%, respectively, in the ad libitum animals. The greater effect of clenbuterol in soleus muscle agrees with results from other workers (e.g., Maltin et al., 1987) and shows that Type I "working muscles" tend to respond better than fast twitch "nonworking muscles," although there are a number of contradictory reports (e.g., Young and McElliot, 1989). The greater responsiveness of soleus muscle may relate to the fact that it contains a very high density of atypical β -adrenoceptors (Roberts et al., 1993; Sillence et al., 1993), and there is some evidence (Cartaña and Stock, 1995) that clenbuterol may affect muscle metabolism via this atypical β -adrenoceptor rather than β_2 -adrenoceptors. The greater anabolic effect of clenbuterol on soleus compared to gastrocnemius muscle was also evident in the

R17C and in the R12C rats. In a study by Choo et al. (1990), in which animals were fed 8 mg of clenbuterol/kg diet at 50% of ad libitum intake (approximately 100% of maintenance), the increase in the mass of gastrocnemius was only 7%. These variable responses suggest that the plane of nutrition may affect the relative proportions of muscle β -adrenoceptor subtypes differently in different muscles.

The weight of all the viscera was reduced by food restriction to both levels (R17 and R12) and, apart from the gastrointestinal tract, clenbuterol had no effect reversing these reductions. Changes in the size of the heart following β -agonist treatment are known to be variable; Emery et al. (1984) found no change in the weight of the heart, whereas Sainz and Wolff (1988) using cimaterol found an increase in heart mass. In the current study, as in that of Reeds et al. (1986) using the same dietary admixture of clenbuterol, there was a significant increase of the weight of the heart in the ad libitum animals, but no change in the weight of liver and kidneys. Generally, these changes in mass reflected the changes in protein content in the viscera, and once again clenbuterol had little or no effect on reductions caused by food restriction. One noticeable exception was shown in the most severely restricted group, in which the gastrointestinal tract exhibited an anabolic response to clenbuterol, with increases in weight and in protein content and the liver with an increase in its protein content. It seems that clenbuterol treatment in the most severely restricted animals may have exerted a protective role in the gastrointestinal tract, and there is some histological evidence (Cardoso, 1993) to suggest that the structural integrity of the small intestine was preserved in the R12 rats receiving clenbuterol compared to the R12 animals. A protective role in the gastrointestinal tract may relate to the presence of an atypical β -adrenoceptor similar, or even identical to, that detected in muscle, and which may correspond to the β_3 -adrenoceptor (Roberts et al., 1993; Sillence et al., 1993).

Clenbuterol is known to exert mild thermogenic effects (Rothwell and Stock, 1984), and these and other workers (Reeds et al., 1988) have found increases of the weight of BAT following chronic treatment in animals given ad libitum access to feed. In this study, there were no effects of clenbuterol on BAT weight, except in the most severely restricted group. It is unlikely that this increase in weight (23%) was due to lipid accumulation, given that the animals had little fat in their carcass or epididymal fat pads, so it might be presumed that the increase in weight was due to protein and/or water. Decreases in carcass lipid following food restriction were pronounced, and not surprisingly clenbuterol had little or no additional effect on this loss of body lipid or on the size of epididymal fat pads in the restrictively fed animals. In the ad libitum rats, the lipid mobilizing

effects of clenbuterol observed by all previous workers were seen.

Food restriction at the lowest level causes a reduction in mass of the skin. Clenbuterol caused a further decrease in the mass of skin in both feed-restricted groups but had no effect in the rats with ad libitum intake. The results from Sainz and Wolff (1988) with clenbuterol-treated rats given ad libitum access to feed also shown unaffected skin weights, and the reductions observed in the feed-restricted groups here were unexpected. One possible interpretation of this effect is that during food restriction the anabolic repartitioning effects of clenbuterol depend on mobilization of protein from the skin in order to support protein deposition in other, more essential, tissues.

Implications

The results from this study demonstrate that the anabolic effects of clenbuterol are sustained in feed-restricted rats and that these effects are potentiated when compared to untreated feed-restricted rats. In particular, muscle and gastrointestinal tract show greater responses to clenbuterol in the feed-restricted rats than in those with ad libitum access to feed. The results from the present study suggest that clenbuterol treatment may have some value in protecting the gastrointestinal tract and off-setting the reductions in live weight gain and muscle in animals subjected to accidental or seasonal interruptions in food supply.

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